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Progesterone Alters Biliary Flow Dynamics

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Objective
To test the hypothesis that progesterone alters sphincter of Oddi and gallbladder function and, therefore, bile flow dynamics.

Summary Background Data
Although the effects of progesterone on the biliary tract have been implicated in the increased incidence of gallstones among women, the specific effects of prolonged elevation of progesterone levels, such as occurs with contraceptive progesterone implants and during pregnancy, on the sphincter of Oddi and biliary flow dynamics are still incompletely understood.

Methods
Adult female prairie dogs were randomly assigned to receive subcutaneous implants containing either progesterone or inactive pellet matrix only. Hepatic bile partitioning and gallbladder emptying were determined 14 days later using ^{99m}Tc-Mebrofenin cholescintigraphy.

Results
Significantly less hepatic bile partitioned into the gallbladder in progesterone-treated than in control animals. The gallbladder ejection fraction was significantly reduced from 73 ± 6% in controls to 59 ± 3% in the progesterone-treated animals. The rate of gallbladder emptying was significantly reduced from 3.6 ± 0.3%/minute to 2.9 ± 0.1%/minute.

Conclusions
Progesterone administered as subcutaneous implants alters partitioning of hepatic bile between gallbladder and small intestine and, therefore, gallbladder filling. Progesterone also significantly impairs gallbladder emptying in response to cholecystokinin. The effects of progesterone on the sphincter of Oddi and the gallbladder may contribute to the greater prevalence of gallstones and biliary motility disorders among women.

Most benign diseases of the biliary tract occur more commonly in women than men.[1] Gallstones are found in approximately 10% of the population, with the incidence in women (14.6%) twice that in men (6.7%).[2] Women are also at increased risk of developing chronic acalculous cholecystitis[3] and sphincter of Oddi dysfunction.[4,5] Epidemiologic studies suggest that current or previous oral contraceptive pill use[6] and parity[7] are significant risk factors for the development of gallstones. These effects may be mediated by known effects of female sex hormones on the biliary tree. Estrogen promotes biliary cholesterol saturation.[1,8] Elevated progesterone levels during pregnancy[9] or in the luteal phase of the menstrual cycle[10] are associated with an increase in resting gallbladder volume and an impaired gallbladder response to cholecystokinin (CCK). Progesterone has also been shown to inhibit contraction of strips of gallbladder muscle[11] *in vitro* and, when administered orally, gallbladder emptying *in vivo*.[12]

However, the specific effects of prolonged elevation of progesterone levels, such as occurs with contraceptive progesterone implants and during pregnancy, on the sphincter of Oddi and biliary flow dynamics are still incompletely understood. The present study was designed to test the hypothesis that progesterone alters sphincter of Oddi and gallbladder function and, therefore, bile flow dynamics.

MATERIALS AND METHODS

Twenty adult female prairie dogs (*Cynomys ludovicianus*), obtained from Otto Martin Locke (New Braunfels, TX) were used in this study. The animals were caged in groups of four in thermoregulated rooms (23°C) with phys-

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iologic sleep/wake cycles. All the animals were maintained on a trace-cholesterol, nonlithogenic diet (Purina Laboratory Chow, Ralston-Purina Co., St. Louis, MO) for ≥1 month before the study. Animal care and procedures were in accordance with the Guidelines for the Ethical Use of Animals as described by the National Institutes of Health and supervised by the Department of Comparative Medicine in The Johns Hopkins Medical Institutions.

Each animal was fasted for 16 hours but allowed water *ad libitum.* General anesthesia was obtained using ketamine (100 mg/kg) and diazepam (500 μg/kg) intramuscularly. Under sterile conditions, through a 1-cm transverse incision, a subcutaneous pocket was created in the interscapular region. The animals were then randomly assigned to have either a slow-release implant (Innovative Research of America, Toledo, OH) containing progesterone (200 mg, n = 10) or a placebo implant consisting of inert pellet matrix only (n = 10) placed in the pocket. The incision was closed using subcuticular polyglycolic acid sutures. The animals were allowed to recover from the anesthesia and returned to the colony. The implants are designed to release their constituents at a controlled rate over varying periods of time, and those used in this study released the entire dose of progesterone over 3 weeks, a dose calculated to raise serum progesterone levels to two to three times baseline.[13]

Gallbladder filling and emptying was determined 14 days later. After another overnight fast with *ad libitum* access to water, the animals were anesthetized using ketamine (100 mg/kg) intramuscularly. A 25-gauge butterfly cannula was placed percutaneously in the long saphenous vein, and anesthesia was maintained thereafter using a continuous intravenous infusion of ketamine (100 mg/kg/hour) and diazepam (0.5 mg/kg/hour). Body temperature was monitored and maintained between 36.5 and 37.5°C with a warming pad.

The animals were positioned under a gamma camera (Trionics XLT system, Bristol, CT) equipped with a parallel beam collimator and connected to a SUN Unix work station. Continuous whole-body imaging (anteroposteror axis) was performed at 1 minute/frame for 1 hour immediately after intravenous administration of 300 μCi of ^{99m}Tc-Mebrofenin (Squibb Diagnostics, Princeton, NJ) at time 0. CCK-octapeptide (Squibb Diagnostics) was then infused at a rate of 5 ng/kg/min for 20 minutes. Imaging continued during the CCK infusion (30 seconds/frame) and for 40 minutes afterwards (1 minute/frame).

Regions of interest were placed on the liver, the gallbladder, the entire small intestine, and the heart (background). Actual counts per frame were corrected for radioactive decay, background activity, and, in the case of the gallbladder, overlapping liver tissue. Time/activity curves were calculated from the corrected data for the gallbladder, liver, and small intestine; a typical time/activity curve in a control animal is shown in Figure 1. The proportions of radiolabel partitioned in the liver, gallbladder, and small intestine were determined during the initial six consecutive 10-minute

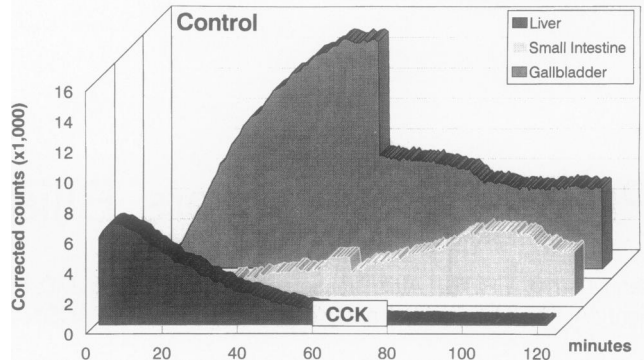

Figure 1. Time/activity curve for a control animal showing activity (vertical axis) in three regions of interest against time (horizontal axis): liver, gallbladder, and small intestine.

periods. In addition, the proportion of gallbladder volume ejected and the rate of gallbladder emptying were calculated. The area of the region of interest representing the gallbladder was also measured as an index of gallbladder volume.

After cholescintigraphy, the animals were allowed to recover and were returned to the colony when radioactivity had declined to background levels. The administration of CCK to stimulate gallbladder emptying during cholescintigraphy profoundly alters gallbladder bile composition.[14–16] To restore a steady state, the hormone implants were replaced at 3 weeks, and 10 days later, after a 16-hour fast, anesthesia was induced using ketamine (100 mg/kg) intramuscularly and a 24-gauge cannula was inserted into the femoral vein. Anesthesia was maintained thereafter with intermittent intravenous infusion of α-chloralose (2 mg/kg/min) alternated with lactated Ringer's solution infused at 0.2 ml/min. Through an upper midline abdominal incision, the extrahepatic biliary tree, duodenum, and stomach were exposed. The cystic duct was isolated, doubly ligated, and divided, and a cholecystectomy was performed. Gallbladder volume was determined by weighing the gallbladder, and the contents were inspected for the presence of cholesterol crystals or early stone formation.

At the termination of the experiment, the animals were killed by exsanguination. Serum was separated by centrifugation of 10 ml of blood at 3000*g* for 10 minutes and frozen at −20°C for later analysis. Serum progesterone was subsequently measured using a competitive binding radioimmunoassay kit (Sigma Chemical Co., St. Louis, MO).

Isotope partitioning into the three compartments (liver, small intestine, gallbladder) is expressed as mean (± standard error) percentage of corrected total counts in the three compartments. The fractions of the total partitioning of bile among the three regions of interest were compared using analysis of variance. Animal weight, gallbladder volumes, ejection fraction, and emptying rate were compared between the two groups using the Student's t test.

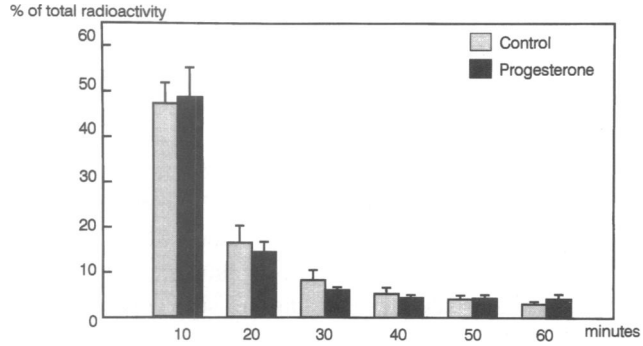

Figure 2. Percentage of total counts (mean ± standard error) partitioned in the liver during each 10-minute time period during the first hour in control and progesterone-treated animals. (*p < 0.05 *vs.* control animals, analysis of variance)

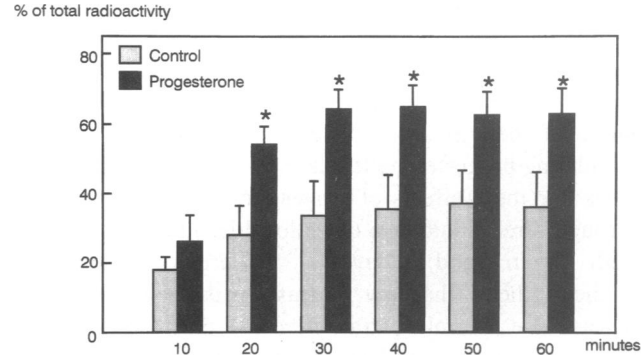

Figure 4. Percentage of total counts (mean ± standard error) partitioned in the entire small intestine during each 10-minute time period during the first hour in control and progesterone-treated animals. (*p < 0.05 *vs.* control animals, analysis of variance)

RESULTS

Animals in both groups maintained a healthy coat of fur and maintained weight similarly during the period of the experiment. At entry into the study, control animals weighed 990 ± 92 g and animals randomized to progesterone treatment weighed 1133 ± 49 g. At completion, control animals weighed 999 ± 64 g and progesterone-treated animals weighed 1125 ± 56 g. The differences between control and progesterone-treated animals and the changes in weight during the study did not differ significantly. Serum progesterone levels were significantly greater in progesterone-treated animals (13.1 ± 1.2 nmol/l) than in control animals (4.6 ± 4.5 nmol/l) ($p < 0.05$, Student's t test).

Isotope clearance by the liver and subsequent excretion of labeled hepatic bile was similar in progesterone-treated and control animals (Fig. 2). However, in the progesterone-treated group, significantly less of the isotope-labeled hepatic bile partitioned into the gallbladder (33 ± 7.4% at 60 minutes) than in control animals (61 ± 10% at 60 minutes) during each of the 10-minute time periods during the first hour ($p < 0.05$) (Fig. 3). Conversely, significantly more of the labeled hepatic bile partitioned into the small intestine in the progesterone-treated animals (63 ± 7% at 60 minutes) than in the control group (36 ± 10% at 60 minutes) during the majority of the 10-minute time periods ($p < 0.05$) (Fig. 4).

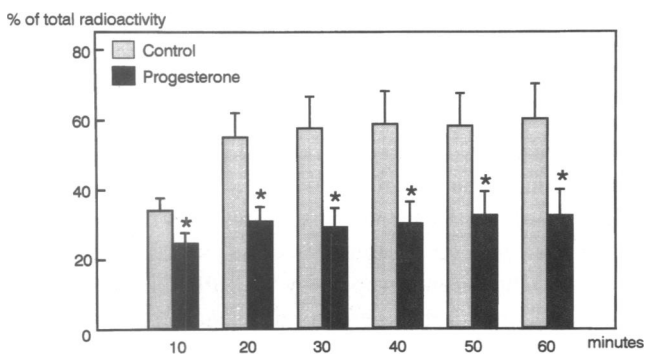

Figure 3. Percentage of total counts (mean ± standard error) partitioned in the gallbladder during each 10-minute time period during the first hour in control and progesterone-treated animals. (*p < 0.05 *vs.* control animals, analysis of variance)

Gallbladder volume as determined by measurement of the area of region of interest did not differ significantly between control (117 ± 20 pixels) and progesterone-treated animals (106 ± 15 pixels). Similarly, when gallbladder volume was determined at laparotomy in the second phase of the study, there was no significant difference between gallbladder volume in control (1.4 ± 0.2 ml) and progesterone-treated animals (1.4 ± 0.1 ml). Gallbladder ejection fraction and emptying rate in response to CCK infusion were significantly reduced by progesterone treatment. Ejection fraction was reduced from 73 ± 6% in controls to 59 ± 3% in the progesterone-treated animals ($p < 0.05$). The rate of emptying was similarly reduced from 3.6 ± 0.3%/minute to 2.9 ± 0.1%/minute ($p < 0.05$). There was no evidence of cholesterol crystals or stone formation in any animals in either group.

DISCUSSION

In this experimental model, progesterone administered as subcutaneous implants resulted in a reduction in the proportion of hepatic bile partitioning into the gallbladder and a corresponding increase in the proportion passing directly into the small intestine. The effects of progesterone were not mediated through direct effects on resting gallbladder volume, hepatic clearance, or excretion of the isotope. Progesterone also significantly inhibits gallbladder emptying, as determined by ejection fraction during cholescintigraphy.

The key components of cholesterol gallstone formation are cholesterol supersaturation, accelerated nucleation, and biliary stasis.[17] Increased serum progesterone levels appear to contribute to cholesterol gallstone formation by promotion of biliary stasis. The presence of detectable levels of progesterone receptors in the wall of the gallbladder has been shown to be associated with significant reductions in gallbladder ejection fraction when compared with receptor-negative gallbladders.[18] During pregnancy, when serum

levels of progesterone are elevated, resting gallbladder volume is increased and ejection fraction in response to a test meal is reduced.[9] Similarly, other investigators have detected impaired gallbladder emptying during the luteal phase of the menstrual cycle,[10] also a time of increased circulating progesterone levels. Experimental evidence suggests that these effects of progesterone are partly mediated through direct inhibition of gallbladder muscle contraction both *in vitro* and *in vivo*.[11,19,20] Further, epidemiologic studies indicate that any increase in the risk of gallstone disease due to oral contraceptive use correlates only with the progesterone component of the contraceptive pill.[21]

The method of administration of progesterone used in this study was specifically chosen because of the similarity to long-acting contraceptive implants recently licensed for use in the United States. Unlike combined oral contraceptive agents, which mimic phasic changes in hormone levels during the menstrual cycle, progesterone implants result in sustained elevation of progesterone levels, creating a hormonal milieu closer to that found during pregnancy,[22,23] which is known to favor stone formation.[24] Even though serum levels may not be as high as during pregnancy, the absence of phasic variation may prevent the gallbladder from recovering, resulting in prolonged stasis, which favors stone formation. Because the present study used only relatively short-term exposure to progesterone, this must remain speculative. However, the observation that small gallstones identified during pregnancy disappear in up to one third of women suggests that gallbladder function may recover after a reduction in progesterone levels.[24] Although the elevation in progesterone levels associated with progesterone contraceptive implants is considerably less than in pregnancy, the longer duration of exposure (up to 5 years) may increase the risk.

The dose of progesterone used in this study produced a threefold rise in serum progesterone levels compared to control animals. This increment is considerably smaller than that seen within the menstrual cycle in women, where progesterone levels rise up to 50-fold from 0.2 to 0.6 ng/ml in the follicular phase to 6.5 to 32.2 ng/ml in the luteal phase.[25] Progesterone levels typically rise to >300 ng/ml in late pregnancy. Although contraceptive progesterone implants such as Norplant (Wyeth Ayerst, Philadelphia, PA) typically yield serum levels of 0.4 to 2.0 ng/ml of levonorgestrel,[26] synthetic progesterones such as levonorgestrel are up to 16 times more potent than pure progesterone.[27] The data from the present study indicate that exposure to these sustained but modest levels of progesterone may have profound effects on biliary motility and mandate studies to determine the magnitude of these effects in humans.

Bile flow dynamics are determined by hepatic excretion, sphincter of Oddi motility (which plays a key role in the regulation of both gallbladder filling and emptying[15]), gallbladder compliance, and gallbladder contraction. Although the effects of progesterone on the gallbladder have been previously characterized, alterations in gallbladder filling or sphincter of Oddi motility caused by progesterone have not previously been described. However, previous studies suggest that biliary motility differs between male and female prairie dogs.[28] Further, estrogen has been shown to have inhibitory effects on sphincter of Oddi motility in this species.[29] In the prairie dog, as in the rabbit and the opossum[30] but unlike the human, sphincter of Oddi phasic wave activity is increased in response to the biliary prokinetic agents.[31] However, the effects of sphincterotomy in virtually abolishing gallbladder filling are similar in the prairie dog[15,16] and in the human,[32] suggesting a similar role of the sphincter of Oddi in regulating this aspect of gallbladder function.

The present study confirms an inhibitory effect of progesterone on gallbladder emptying[11,19,20] and suggests that progesterone has even more profound effects on biliary flow dynamics. Similar values for clearance and hepatic secretion of circulating ^{99m}Tc-Mebrofenin between the groups indicate no effects of progesterone on this component of biliary dynamics. However, progesterone treatment results in a significant diversion of hepatic bile away from the gallbladder and into the small intestine. This effect of progesterone on bile flow could reflect altered basal sphincter of Oddi motility, an increase in resting gallbladder volume preventing accommodation of hepatic bile, a reduction in gallbladder compliance, or a combination of these factors. However, measurement of gallbladder volume indicates no difference between the groups, suggesting that the effects of progesterone on sphincter of Oddi motility are more likely to be responsible for the differences in hepatic bile partitioning observed between the two groups in this study. However, further studies to evaluate the influence of progesterone on the sphincter of Oddi will be required to confirm and quantify this effect.

In conclusion, the results of this study demonstrate that progesterone administered as subcutaneous implants significantly alters both partitioning of hepatic bile between the gallbladder and the small intestine and gallbladder emptying in response to CCK stimulation, thus promoting biliary stasis. Altered bile partitioning is likely to result from a direct effect of progesterone on the sphincter of Oddi. Although gallbladder bile is normally quite unsaturated in the prairie dog, gallbladder bile is frequently saturated with cholesterol in the human. Because biliary stasis is also recognized a key event in gallstone formation,[17] the novel effects of progesterone on gallbladder filling found in this study and the previously known effects on gallbladder emptying may both contribute to the greater propensity for gallstone formation in women. Further, these effects of progesterone on biliary flow dynamics raise concern regarding the risk of gallstone disease in those exposed to prolonged and sustained levels of progesterone, such as occurs with contraceptive progesterone implants.

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